

Table S1. Summary of off-targets predicted by CasOffinder.

gRNA	Target Sequence	Gap	Mismatch	# off-targets predicted
crRNA1	TTTNGTCACAGATCACAAACTTCAAAT	0	4	1
crRNA1	TTTNGTCACAGATCACAAACTTCAAAT	0	5	10
crRNA1	TTTNGTCACAGATCACAAACTTCAAAT	-1	1	3
crRNA1	TTTNGTCACAGATCACAAACTTCAAAT	-1	2	1
crRNA1	TTTNGTCACAGATCACAAACTTCAAAT	-1	3	5
crRNA1	TTTNGTCACAGATCACAAACTTCAAAT	-1	4	74
crRNA1	TTTNGTCACAGATCACAAACTTCAAAT	-1	5	1099
crRNA1 Total				1193
crRNA2	TTTNAACAAGTGGGGCGCAGATCCTGAG	-1	2	1
crRNA2	TTTNAACAAGTGGGGCGCAGATCCTGAG	-1	3	3
crRNA2	TTTNAACAAGTGGGGCGCAGATCCTGAG	-1	4	14
crRNA2	TTTNAACAAGTGGGGCGCAGATCCTGAG	-1	5	155
crRNA2 Total				173
gRNA1	ACAGATCACAAACTTCAAATGNGG	0	2	1
gRNA1	ACAGATCACAAACTTCAAATGNGG	0	4	19
gRNA1	ACAGATCACAAACTTCAAATGNGG	0	5	249
gRNA1	ACAGATCACAAACTTCAAATGNGG	-1	1	1
gRNA1	ACAGATCACAAACTTCAAATGNGG	-1	2	3
gRNA1	ACAGATCACAAACTTCAAATGNGG	-1	3	69
gRNA1	ACAGATCACAAACTTCAAATGNGG	-1	4	1083
gRNA1	ACAGATCACAAACTTCAAATGNGG	-1	5	17604
gRNA1 Total				19029
gRNA2	AAGTGGGCGCAGATCCTGAGNGG	0	2	12
gRNA2	AAGTGGGCGCAGATCCTGAGNGG	0	3	357
gRNA2	AAGTGGGCGCAGATCCTGAGNGG	0	4	3699
gRNA2	AAGTGGGCGCAGATCCTGAGNGG	0	5	2255
gRNA2	AAGTGGGCGCAGATCCTGAGNGG	-1	1	13
gRNA2	AAGTGGGCGCAGATCCTGAGNGG	-1	2	378
gRNA2	AAGTGGGCGCAGATCCTGAGNGG	-1	3	4205
gRNA2	AAGTGGGCGCAGATCCTGAGNGG	-1	4	9280
gRNA2	AAGTGGGCGCAGATCCTGAGNGG	-1	5	51054
gRNA2 Total				71253

Activities and specificities of CRISPR-Cas9 and Cas12a nucleases for targeted mutagenesis in maize

Table S2. Oligonucleotides used in this study.

Forward primer	Sequence	Reverse primer	Sequence	Sequencing primer	Sequence
On-target analysis					
Zm-g12-F2	CACAGCCTTGCAATCAATTC	Zm-g12-R2	GCTGACGTGGAAGGAGTAGC	ZmG12-exon2-F1	ACACCGTGTCTTCGTCAAAA
ZmG12-exon2-F1	ACACCGTGTCTTCGTCAAAA	ZmG12-exon2-R1	GACCAGCCACAGGTCTC	ZmG12-exon2-F1	ACACCGTGTCTTCGTCAAAA
Cas9-gRNA1 off-target analysis					
CIRCLE-seq					
CIRgRNA1-OFF1F	AGTGACTACATGGGGCAAC	CIRgRNA1-OFF1R	CTGGCGATGTCGATCTCTTT	CIRgRNA1-OFF1seq	CACTATCGCCCCCTTGATTA
CIRgRNA1-OFF2F	GAGCACGGTGACGCTATGTA	CIRgRNA1-OFF2R	TTCGAAAGGATGTCAATCTGG	CIRgRNA1-OFF2seq	CTCTCACTTGCTGGCAAAA
CIRgRNA1-OFF4F	TGGGGAGACTGAACATAGCA	CIRgRNA1-OFF4R	AGTGGTCCAGTCCCAACAG	CIRgRNA1-OFF4seq	TGCATATCCGACCAGAAACA
CIRgRNA1-OFF5F	GAATGTCAGACTCGCCTTCC	CIRgRNA1-OFF5R	TCGCTGCTTCTGTCTCTAC	CIRgRNA1-OFF5seq	CACTTCCCCTAAAGCAAGCA
CIRgRNA1-OFF6F	GAATGCGGATGCTTTTGT	CIRgRNA1-OFF6R	GAGCTTTTGGGTTGGAGTT	CIRgRNA1-OFF6seq	TATGGTCTGCCATCTTTT
CIRgRNA1-OFF7F	TGGGAATTGGTTTCTCTTG	CIRgRNA1-OFF7R	GGCATGGAACTGTCTTGCT	CIRgRNA1-OFF7seq	TGGGAATTGGTTTCTCTTG
CIRgRNA1-OFF8F	AAACCCACGCTTCTGTAAA	CIRgRNA1-OFF8R	CTCCGCTAGCACTGAATC	CIRgRNA1-OFF8seq	GAATCGCCATGTAGAGCA
CIRgRNA1-OFF9F	AATTGGAGGGAAGGCTAGGA	CIRgRNA1-OFF9R	GAACCAAATGCGGAACTGT	CIRgRNA1-OFF9seq	TAGGATGGGAATGCTTGAGG
CIRgRNA1-OFF10F	AAGCAACCAACTGGCACTGT	CIRgRNA1-OFF10R	GGCAAGCGGTATTTTATCA	CIRgRNA1-OFF10seq	TGGCACTGTTTATCTGTGC
CIRgRNA1-OFF11F	CCTTTCAGATCGTCCTTTC	CIRgRNA1-OFF11R	TTGGTGTTCCTCTCCATC	CIRgRNA1-OFF11seq	GCCCCTAGCAAGAACCTTTT
CIRgRNA1-OFF12F	CCCGAAAGAAGGCTTACTA	CIRgRNA1-OFF12R	GTTTTGTACCGGCTAACG	CIRgRNA1-OFF12seq	ATTCCAACAGCTTGCTCCAC
CIRgRNA1-OFF13F	AGAGTGCCAAGGACAACACC	CIRgRNA1-OFF13R	TTGAGCAACCCTAAGCAAGG	CIRgRNA1-OFF13seq	CGTGCCATTTATGAGTCCAA
CIRgRNA1-OFF14F	ACACAAAGAATGCGGGTGAT	CIRgRNA1-OFF14R	GGTTGGACCGTTGGAGACTA	CIRgRNA1-OFF14seq	TGATTTGTTTCCGAGGTTT
CIRgRNA1-OFF16F	TCCATTCCATTGATTGCTGA	CIRgRNA1-OFF16R	GGCCCTTCATGATCAGTGT	CIRgRNA1-OFF16seq	GGAGAACCGTGTCTGTCTT
CIRgRNA1-OFF17F	AAATGCTGGCCAAATTGTCT	CIRgRNA1-OFF17R	GTCCACCCATGCCTGATACT	CIRgRNA1-OFF17seq	GGCCAAATTGTCTGAAAAGG
Cas-OFFinder					
CASgRNA1-OFF1F	GCAGGTTGGGAGACTGAAC	CASgRNA1-OFF1R	CAACCTACTGAACGGACCG	CASgRNA1-OFF1seq	GCAGGTTGGGAGACTGAAC
CASgRNA1-OFF2F	GGACAGTTTTTCCCACCCCT	CASgRNA1-OFF2R	CCTTCGTCGAGCAAGCTACA	CASgRNA1-OFF2seq	GGACAGTTTTTCCCACCCCT
Cas9-gRNA2 off-target analysis					
CIRCLE-seq					
CIRgRNA2-OFF3F	TTTTACCCACGCAGTCCAGT	CIRgRNA2-OFF3R	AGCCCTGACAAGGACGTCTA	CIRgRNA2-OFF3seq	TTCCTTTGTCTCTCTCAGCA
CIRgRNA2-OFF7F	GTGCCAAAAGCAGCTTATCC	CIRgRNA2-OFF7R	TGCATATAGCGCAAAGATG	CIRgRNA2-OFF7seq	GCCTTTGCTTGTGCGATAAT
CIRgRNA2-OFF8F	TTGGTTGAGAGGGTTTCAGC	CIRgRNA2-OFF8R	CGGTCCATACCATGACACAG	CIRgRNA2-OFF8seq	GGTCCGTTTGAGATTGAGAA

Activities and specificities of CRISPR-Cas9 and Cas12a nucleases for targeted mutagenesis in maize

Table S2. Oligonucleotides used in this study.

Forward primer	Sequence	Reverse primer	Sequence	Sequencing primer	Sequence
CIRgRNA2-OFF12F	GACCACTTCCCACTCACAGA	CIRgRNA2-OFF12R	ACCCAGAAAGGGAAAAGGAG	CIRgRNA2-OFF12seq	GTGTCCTCGCGCAACACTA
CIRgRNA2-OFF13F	CATCGCGCCTTCTCATCTAT	CIRgRNA2-OFF13R	CTGGTGCTCTTTCCCTCTG	CIRgRNA2-OFF13seq	TAATGAGTGGGACCCCTGTGG
CIRgRNA2-OFF14F	CTTCTTCTTGCCACCAGAC	CIRgRNA2-OFF14R	GTCGGCGATGTAGGTCGATA	CIRgRNA2-OFF14seq	GCACCTGCATCACCTAGCC
CIRgRNA2-OFF15F	CCTTCCCTCTTCTGGTCTCC	CIRgRNA2-OFF15R	AGGATCCGCATCTCCTTCTT	CIRgRNA2-OFF15seq	CGCTCTCAGATCCCTCTCC
CIRgRNA2-OFF16F	TTTATCAGCTTCGGGTCGAT	CIRgRNA2-OFF16R	GAAAGCTCCCTCGGAAACTC	CIRgRNA2-OFF16seq	GCTCAAGCCACGATGTATCA
CIRgRNA2-OFF17F	GCCTGCTTTAGCTCGCTATG	CIRgRNA2-OFF17R	TCAAGTTCTCCCGTGGAGTT	CIRgRNA2-OFF17seq	CATACTTGCCCGATCCTAT
CIRgRNA2-OFF19F	CCAAAAGGGGTGGAGTTTTA	CIRgRNA2-OFF19R	CCGTTTACGTAGGAGCATT	CIRgRNA2-OFF19seq	TGGCCAGGCCTATTGTGTTA
CIRgRNA2-OFF21F	TCTGATGATGGCATTCTCGA	CIRgRNA2-OFF21R	GCTTTAGCACCTGGAAAGCA	CIRgRNA2-OFF21seq	GAGAAGCATTGTTTCATTTGC
CIRgRNA2-OFF22F	GACGGTGTACGGCAACAA	CIRgRNA2-OFF22R	TTGTGCTTGTGTGATGGTG	CIRgRNA2-OFF22seq	CGTCGACTTTCTCCGTGAAG
CIRgRNA2-OFF23F	CTGGCATTGCACAGTCGTAT	CIRgRNA2-OFF23R	GTCGCAAATCCTTGATCCAC	CIRgRNA2-OFF23seq	AGTCGTATCGTGGTCTCTCG
CIRgRNA2-OFF24F	CAGGGTTATTGGTGGGAGTG	CIRgRNA2-OFF24R	ACTACTCGCGGATGGAGTTG	CIRgRNA2-OFF24seq	AAGAGGGACCATCTGCTGTG
CIRgRNA2-OFF25F	GGGAGTGTACGCAACTTCCTA	CIRgRNA2-OFF25R	TGGCTGCTTGTACTGTTCAC	CIRgRNA2-OFF25seq	TGGGGTATTTGGTTTCATCC
CIRgRNA2-OFF28F	GGTTTGCAGAGGTGGTACT	CIRgRNA2-OFF28R	CCAGGATGGCTATGAAGCTC	CIRgRNA2-OFF28seq	GTGAAGTCCCTGGACGAATG
CIRgRNA2-OFF29F	TTTGCAGCCTCGTCTGTCTA	CIRgRNA2-OFF29R	TCCCAAGTCCACACTCTCCT	CIRgRNA2-OFF29seq	CAACCATGCCACATGAGTA
CIRgRNA2-OFF32F	AATTTTGGTTTCTCGGATCG	CIRgRNA2-OFF32R	ATACCCCTCCGTTTTGTCTT	CIRgRNA2-OFF32seq	TCGGATCGCGTGATACTACA
CIRgRNA2-OFF36F	GTCTTACGACGGTCACCA	CIRgRNA2-OFF36R	CAGGTCACCAGCTTCAAGTGT	CIRgRNA2-OFF36seq	GTGACGTGGAAGGAGAAGGA
CIRgRNA2-OFF38F	GCTAATAATTTCCCGCAACG	CIRgRNA2-OFF38R	TGGATAGACTGGACTGGATGG	CIRgRNA2-OFF38seq	GAGACCGACGGACCTGTAAA
CIRgRNA2-OFF39F	CTCCAGAGGGAGGACAAATG	CIRgRNA2-OFF39R	CCGTCGTTAGGCAAAAACAC	CIRgRNA2-OFF39seq	AGGGAGGACAAATGCCAAG
CIRgRNA2-OFF41F	AAACCTGACCCTGATCCAAA	CIRgRNA2-OFF41R	AGTAGAAATCCGGCGAACCT	CIRgRNA2-OFF41seq	TCCCTGCTGCAATGTC
CIRgRNA2-OFF46F	AGCTCAGGTACACGCTGCTC	CIRgRNA2-OFF46R	CGGGTCTTTCTGGTCTTCAA	CIRgRNA2-OFF46seq	GAGCTCAAACGCTTCGCTAT
CIRgRNA2-OFF49F	TCACGAAAGGAACGAAAGGT	CIRgRNA2-OFF49R	TTTTCCATGGAGCCAGATTC	CIRgRNA2-OFF49seq	CGATGAGCACCAGTCATTGT
CIRgRNA2-OFF54F	GTGTCCCTGTTTCCATGACC	CIRgRNA2-OFF54R	ACATAGCCCTCCCTTAGCAT	CIRgRNA2-OFF54seq	GGGTGTTAGGGAGGGAGAAG
Cas-OFFinder					
CASgRNA2-OFF1F	TAGGCAGACAATGAGCCAAA	CASgRNA2-OFF1R	TAGATCGAGAAGGCGTCTCTG	CASgRNA2-OFF1seq	CCAAAATAACCTGAGCGATCT
CASgRNA2-OFF4F	CCGAGATAAGACACTGACG	CASgRNA2-OFF4R	GTGACGTGGAAGGAGAAGGA	CASgRNA2-OFF4seq	CAAGACGACGTGTTCTGCTAA
T-DNA screening					
zCas9-F	CCGATTCTGGAGAAGATGGA	zCas9-R	TCGAAGAGATGGGCGTAAGT		
LbCas12a-F	AATGGAACCGGGAGTATGAC	LbCas12a-R	ACATGTCGCCCTTCTTGAAC		

Activities and specificities of CRISPR-Cas9 and Cas12a nucleases for targeted mutagenesis in maize

Table S2. Oligonucleotides used in this study.

Forward primer	Sequence	Reverse primer	Sequence	Sequencing primer	Sequence
Vector construction					
LNK-165-F-NEW	CATGGGGTACCCAATTGTTCCGGAACCTCTAGATAAGCTTA				
LNK-165-R-NEW	CCGGTAAGCTTATCTAGAGTTCGGAACAATTGGGTACCC				
zCas9-F-NcoI	TGGACCATGGATTACAAGGACCACGACGGGGATTA				
zCas9-R-SacI	GTCGAAACCGATGATACGAACGAA				
ZmGL2-crRNA1-F	TAGATGTCACAGATCACAAACTTCAAAT				
ZmGL2-crRNA1-R	GGCCATTTGAAGTTTGTGATCTGTGACA				
ZmGL2-crRNA2-F	TAGATAACAAGTGGGCGCAGATCCTGAG				
ZmGL2-crRNA2-R	GGCCCTCAGGATCTGCGCCCACTTGTTA				
ZmGL2-gR3-F	TGGCACAGATCACAAACTTCAAATG				
ZmGL2-gR3-R	AAACCATTTGAAGTTTGTGATCTGT				
ZmGL2-gR4-F	TGGCAAGTGGGCGCAGATCCTGAG				
ZmGL2-gR4-R	AAACCTCAGGATCTGCGCCCACTT				
CIRCLE-seq					
oSQT1288	P-CGGTGGACCGATGATCUATCGGTCCACCG*T				*indicates a phosphorothioate linkage

Activities and specificities of CRISPR-Cas9 and Cas12a nucleases for targeted mutagenesis in maize

Table S3. Summary of genotyping results of T0 and T1 lines.

Construct	Event	Plant	T0 genotype		Method	#clone	Genotype of T1 progenies (pollen donor was wild-type)	Method	Off-target mutations
A842B	1	1	WT	(0, 0)	Cloning	7			
A842B	1	2	WT	(0, 0)	Cloning	7	(0, 0) x 7	TIDE	
A842B	1	3	Heterozygous	(0, -9)	Cloning	2, 6			
A842B	1	4	Mosaic	(0, -7, -9)	Cloning	5, 1, 2	(0,-9), (+1, -8)	TIDE	
A842B	1	5	Heterozygous	(0, -73)	Cloning	7, 1		TIDE	
A842B	2	1	Heterozygous	(0, -11)	TIDE			TIDE	
A842B	2	2	Heterozygous	(0, -12+1)	TIDE		(+1, -10) x 3, (0, -11) x 4	TIDE	
A842B	2	3	Heterozygous	(0, -9)	TIDE				
A842B	2	4	WT	(0, 0)	TIDE				
A842B	2	5	Heterozygous	0/-11	TIDE				
A842B	3	1	WT	(0, 0)	TIDE				
A842B	3	2	WT	(0, 0)	TIDE				
A842B	3	3	WT	(0, 0)	TIDE				
A842B	3	4	WT	(0, 0)	TIDE				
A842B	3	5	WT	(0, 0)	TIDE				
A842B	5	1	Heterozygous	(0, +1)	TIDE		(+1, -6), (+2, -7), (+1, -8), (0, -7) x 2, (0, -9) x 2	TIDE	
A842B	5	3	Mosaic	(+1, -8, -9)	TIDE				
A842B	5	4	Heterozygous	(0, +1)	TIDE				
A842B	5	5	Biallelic	(-6, -9)	TIDE				
A842B	5	7	Heterozygous	(0, -9)	TIDE				
A843B	1	1	WT	(0, 0)	Cloning	7			
A843B	1	2	WT	(0, 0)	Cloning	8			
A843B	1	3	WT	(0, 0)	Cloning	8			
A843B	1	4	WT	(0, 0)	Cloning	5			
A843B	2	1	WT	(0, 0)	Cloning	8			
A843B	2	2	WT	(0, 0)	Cloning	8	(0, 0) x 7	TIDE	
A843B	2	3	WT	(0, 0)	Cloning	8			
A843B	2	4	WT	(0, 0)	Cloning	8			
A843B	2	5	WT	(0, 0)	Cloning	6			
A843B	3	1	WT	(0, 0)	Cloning	5			
A843B	3	2	WT	(0, 0)	Cloning	5	(0, 0) x 7	TIDE	
A843B	3	3	WT	(0, 0)	Cloning	7			
A843B	3	4	WT	(0, 0)	Cloning	3			
A843B	3	5	WT	(0, 0)	Cloning	7			
A843B	3	6	WT	(0, 0)	TIDE				
A843B	4	1	WT	(0, 0)	TIDE				
A843B	4	2	WT	(0, 0)	TIDE				
A843B	4	3	WT	(0, 0)	TIDE				
A843B	4	4	WT	(0, 0)	TIDE				
A843B	4	5	WT	(0, 0)	TIDE				
A843B	4	6	WT	(0, 0)	TIDE				
A843B	6	1	WT	(0, 0)	TIDE				
A843B	6	2	WT	(0, 0)	TIDE				
A844B	1	1	Heterozygous	(0, -7)	TIDE				
A844B	1	2	Homozygous	(-7, -7)	TIDE				
A844B	1	3	Homozygous	(-7, -7)	TIDE				
A844B	1	4	Homozygous	(-7, -7)	TIDE		(+2, -7), (-7, -6), (-1, -7), (0, -7)	TIDE	NO
A844B	1	5	Homozygous	(-7, -7)	TIDE				
A844B	2	1	Homozygous	(+1, +1)	TIDE				
A844B	2	2	Homozygous	(+1, +1)	TIDE				
A844B	2	3	Homozygous	(+1, +1)	TIDE				
A844B	2	4	Homozygous	(+1, +1)	TIDE				
A844B	2	5	Homozygous	(+1, +1)	TIDE		(0,+1) x3	TIDE	NO
A844B	3	2	Homozygous	(-2, -2)	TIDE		(-2,-1), (-2,-5), (-2,-6), (-2,-2), (-2, +1)	TIDE	NO
A844B	3	4	Homozygous	(-2, -2)	Cloning	8	(-2,+1) x 3, (-2,-2)	TIDE	NO
A844B	3	5	Homozygous	(+1, +1)	Cloning	6			

Activities and specificities of CRISPR-Cas9 and Cas12a nucleases for targeted mutagenesis in maize

Construct	Event	Plant	T0 genotype	Method	#clone	Genotype of T1 progenies (pollen donor was wild-type)	Method	Off-target mutations	
A844B	5	1	Heterozygous	(0, +1)	TIDE				
A844B	5	2	Biallelic	(+1, -6)	TIDE	(+1, +1) x 2, (+1, -2), (0, -6)	TIDE		
A844B	6	1	Heterozygous	(0, +1)	TIDE	(-1, -7), (-7, -7), (0, 0)	TIDE	NO	
A844B	6	2	Heterozygous	(0, +1)	TIDE				
A844B	6	3	Heterozygous	(0, +1)	TIDE				
A844B	6	4	Heterozygous	(0, +1)	TIDE				
A844B	6	5	Heterozygous	(0, +1)	TIDE				
A844B	7	1	Heterozygous	(0, +1)	TIDE				
A844B	7	2	Heterozygous	(0, +1)	TIDE	(+1, -1), (+1, -3), (+1, +1)x2, (0, +1)	TIDE	NO	
A844B	8	1	Biallelic	(+1, -2)	TIDE				
A844B	8	2	Biallelic	(+1, -2)	TIDE	(-2, -5), (-1, -4), (0, +1), (0, -2)x3	TIDE	NO	
A844B	8	3	Heterozygous	(0, +1)	TIDE				
A844B	8	4	Biallelic	(+1, -2)	TIDE				
A844B	8	5	Biallelic	(+1, -2)	TIDE				
A845B	1	1	WT	(0, 0)	Cloning	8			
A845B	1	2	WT	(0, 0)	Cloning	6			
A845B	2	1	Homozygous	(-6, -6)	Cloning	5			
A845B	2	2	Biallelic	(-6, -4+1)	Cloning	6, 2			
A845B	2	3	Biallelic	(-6, -4+1)	Cloning	4, 3			
A845B	2	4	Biallelic	(-6, -4+1)	Cloning	4, 4	(-2, -5), (0, -6), (0, -3) x 2	TIDE	NO
A845B	2	5	Biallelic	(-6, -4+1)	Cloning	5, 3			
A845B	3	1	Biallelic	(-2, -5)	Cloning	4, 4			
A845B	3	2	Biallelic	(-2, -5)	Cloning	1, 5			
A845B	3	3	Biallelic	(-2, -5)	Cloning	5, 3	(-2, -2), (-2, -13), (-2, -15), (-2, -24), (-5, -5), (-5, -24), (-5, -27)	TIDE	NO
A845B	3	4	Biallelic	(-2, -5)	Cloning	1, 7	(-2, -2), (-5, -1)	TIDE	NO
A845B	3	5	Biallelic	(-2, -5)	Cloning	4, 3			
A845B	3	6	Biallelic	(-2, -5)	Cloning	5, 3			
A845B	4	1	Biallelic	(-2, -4)	Cloning	10, 9	(-4, -1) x 2, (-4, -5), (0, -4)	TIDE	NO
A845B	4	3	Biallelic	(-2, -4)	Cloning	4, 2	(-2, -2), (-4, -1), (-4, +4), (0, -4)x2	TIDE	NO
A845B	4	4	Biallelic	(-2, -4)	Cloning	2, 4			
A845B	5	1	Biallelic	(-2, -3)	Cloning	4, 2			
A845B	5	2	Biallelic	(-2, -3)	Cloning	5, 3			
A845B	5	3	Biallelic	(-2, -3)	Cloning	1, 5	(-1, -4), (-3, -9), (-3, -16), (-3, -6), (0, -3)	TIDE	NO
A845B	6	1	Homozygous	(-6, -6)	Cloning	8			
A845B	6	2	Homozygous	(-6, -6)	Cloning	8			
A845B	6	3	Homozygous	(-6, -6)	Cloning	8			
A845B	6	4	Homozygous	(-6, -6)	Cloning	6	(-1, -6)x2, (0, -6)	TIDE	NO
A845B	6	5	Homozygous	(-6, -6)	Cloning	7			
A845B	7	1	Biallelic	(-1, -6)	TIDE				
A845B	7	3	Biallelic	(-1, -6)	TIDE	(-1, -1), (-2, +1), (-1, -5), (-1, -6)	TIDE	NO	
A845B	8	1	Heterozygous	(0, -6)	Cloning	7, 1			
A845B	8	2	Heterozygous	(0, -4)	Cloning	7, 1			
A845B	8	3	WT	(0, 0)	Cloning	8	(-7, -20), (0, -4), (0, 0)x2	TIDE	NO
A845B	8	5	Heterozygous	(0, -3)	Cloning	6, 1			
A845B	10	2	Heterozygous	(0, -6)	TIDE	(0, -6), (0, 0)	TIDE	NO	

Activities and specificities of CRISPR-Cas9 and Cas12a nucleases for targeted mutagenesis in maize

Table S4. List of off-targets identified by CIRCLE-seq and Cas-OFFinder.

Off-target	Chr.	OFF-target position	Strand	# mis-match es	Gap	CIRCLE-seq read	Off-target sequence	F primer	R primer	Sequencing primer	Sequencing ID
Cas12a-crRNA1: CasOFFinder											
1	CAScrRNA1-OFF1	chr2	16677669	-	3	-1	TTTCTTCCAGATCATAAAATTCAAAT	CACTATCGCCCCCTTGATTA	CTGGCGATGTCGATCTCTTT	CACTATCGCCCCCTTGATTA	gRNA1-OFF1
Cas9-gRNA1: CIRCLE-seq											
1	CIRgRNA1-OFF1	chr8	74971851	-	2	-1	754 A-AGATCACAAAGCTCAAATGAGG	CACTATCGCCCCCTTGATTA	CTGGCGATGTCGATCTCTTT	CACTATCGCCCCCTTGATTA	gRNA1-OFF1
2	Target	chr2	8112521	-	0		672 ACAGATCACAAACTTCAAATGCCG	CACAGCCTTGCAATCAATTC	GCTGACGTGAAGGAGTAGC	ACACCGTGCTTCGTCAAAA	
3	CIRgRNA1-OFF2	chr10	41964030	-	6		236 GTTTAACACAAACTTCAAATTTGG	TTGATGGTCATCAGTTTGGTG	TTCGAAAGGATGCAATCTGG	TTGATGGTCATCAGTTTGGTG	gRNA1-OFF2
4	CIRgRNA1-OFF3	chr2	143072090	+	6		80 TTTGAACACGAACTTCAATGGGG	NA	NA	NA	
5	CIRgRNA1-OFF4	chr3	107726662	+	3	-1	60 ACAATCATAAACTTCAA-TGTGG	TTGAAAACCATCCTGGTGCT	TCTTGACAGGACATAGCAAA	TTGAAAACCATCCTGGTGCT	gRNA1-OFF3
6	CIRgRNA1-OFF5	chr3	108808715	+	2		56 ACAGATCACAAAGTCAAATGTGG	GAATGTCAGACTCGCCTTCC	TCGCTGCTTCTGCTGTCTAC	CACCTCCCTAAAGCAAGCA	gRNA1-OFF4
7	CIRgRNA1-OFF6	chr3	1731070	-	3	-1	44 ATAGACCACAA-CTTCAAATGGG	GAATGCGGATGCTTTTGTTT	GAGCCTTTTGGGTTGGAGTT	TATGGGTCGTGCCATCTTTT	gRNA1-OFF5
8	CIRgRNA1-OFF7	chr1	34341100	-	4		36 TCAGATTACAAATTTCAAATGAGG	TGGAAATTTGGTTTCTCTTTG	GGCATGGAAACTGTCTTGCT	TGGGAATTTGGTTTCTCTTTG	gRNA1-OFF6
9	CIRgRNA1-OFF8	chr4	103904220	-	3	-1	30 AAAAAATCACAAACTTCAATA-GGGG	TGGTAAACAAACCGGGTCTAA	CTCCCGCTAGCACTTGAATC	CTCCCGCTAGCACTTGAATC	gRNA1-OFF7
10	CIRgRNA1-OFF9	chr10	52293332	+	7		28 AACGATCAAAAACCTTCAATATGGT	CCCACAGCCCCACTAATACA	CATGCAATTGAGCTGGAGAA	CCCACAGCCCCACTAATACA	gRNA1-OFF8
11	CIRgRNA1-OFF10	chr2	119407415	+	3	1	24 CGAAATCACAAAGTCCAATGGGG	TTTGAAACATTGCAGCATGA	TCAAATGCAGCAGGTCAGTC	TTTGAAACATTGCAGCATGA	gRNA1-OFF9
12	CIRgRNA1-OFF11	chr8	72728483		7		24 TTTAAACACAAACTTCAATTTGGG	GCCCTTAGCAAGAACCTTTT	CACCATTCCCGCACTCTAAT	GCCCTTAGCAAGAACCTTTT	gRNA1-OFF10
13	CIRgRNA1-OFF12	chr5	134878299	-	4	-1	50 ATCAATCACAAA-TTCAAATATGG	CCCAGAAAGAGGCTTCACTA	GTTTTTGATCCCGCCTAACG	ATTCCAAACAGTTGCTCCAC	gRNA1-OFF13
14	CIRgRNA1-OFF13	scaffold5.1434	9927	+	7		154 TTTAATCACAAACTTCAATATGGG	AGAGTCCAAAGGACAACACC	TTGAGCAACCTTAAGCAAGG	CGTGCCATTTATGAGTCCAA	gRNA1-OFF14
15	CIRgRNA1-OFF14	scaffold3.573	33430	-	3	-1	132 AATGATCACAACTTCAAATGTGG	ACACAAAGAAATGCGGGTGAT	GGTTGGACCGTTGGAGACTA	TGATTTGTTTCCCGAGGTTT	gRNA1-OFF15
16	CIRgRNA1-OFF15	scaffold7.852	31292	-	5		124 TTTGAACACAAACTTCAATGGGG	NA	NA	NA	
17	CIRgRNA1-OFF16	scaffold9.645	123031	-	5		70 TGACATCACAAATTTCAAATTTGG	TCCATTCATTGATTGCTGA	GGCCCTTCATGATCAGTGTT	GGAGAACCGTGGTCTGTCTT	gRNA1-OFF16
18	CIRgRNA1-OFF17	scaffold4.2924	93708	-	5		28 TCCTATCACACACTTCAAATGGAG	AAATGTGGCCAAATTTGCT	GTCCACCATGCTGATACT	GGCCAAATTTGCTGAAAAGG	gRNA1-OFF17
19	CIRgRNA1-OFF18	scaffold1.1776	51589	+	6		26 AATCTCACAGCTTCCAATGGGG	NA	NA	NA	
Cas9-gRNA1: Cas-OFFinder											
1	CASgRNA1-OFF1	chr3	107726661	+	3	-1	ACAATCATAAACTTGAATGTGG	GCAGGTTGGGGAGACTGAAC	CAACCTACTGAACGGGACCG	TGCATATCCGACCAGAAACA	gRNA1-OFF11
2	CASgRNA1-OFF2	chr3	123022309	+	3	-1	ACAGTCACACACTTCCAAGAGG	GGACAGTTTTTCCACCCCT	CCTTCGTGAGCAAGCTACA	GCACCACCAAGAGACCAAT	gRNA1-OFF12
Cas9-gRNA2: CIRCLE-seq											
1	CIRgRNA2-OFF1	chr9	13588581	+	6		342 CGTTGGGCCAGATCCAGAGGCG	NA	NA	NA	
2	CIRgRNA2-OFF2	chr7	89451810	+	6		294 CGTTGGGCCAGATCCAGAGGCG	NA	NA	NA	
3	CIRgRNA2-OFF3	chr1	141204025	-	4	-1	278 CGGTGGCAGCAGATCCTGTGG	TTTTACCCACGAGTCCAGT	AGCCCTGACAAGGACGTCTA	TTCTTTGTCTCTCTCAGCA	gRNA2-OFF1
4	CIRgRNA2-OFF4	chr5	97442353	+	4	-1	252 AA-TGGACACAGATCATGAGGAG	NA	NA	NA	
5	CIRgRNA2-OFF5	chr10	20775888	+	4	-1	190 AA-TGGACACAGATCATGAGGAG	NA	NA	NA	
6	CIRgRNA2-OFF6	chr4	3175346	-	4		176 AAGCGGCGTAGCTCCAGAGGGG	NA	NA	NA	
7	CIRgRNA2-OFF7	chr4	111140164	+	5		158 TTGTAGTCGAGATCCTGAGCAG	GTGCCAAAAGCAGCTTATCC	TGCATATAGCCCAAAGATG	GCCTTTGCTTGTGCGATAAT	gRNA2-OFF2
8	CIRgRNA2-OFF8	chr9	77561370	+	5		154 CAGTAGGTGCAGATCCTGAGGAT	TTGGTTGAGAGGGTTTCAGC	CGGTCCATACCATGACACAG	GGTCCGTTTGGATTTGAGAA	gRNA2-OFF3

Activities and specificities of CRISPR-Cas9 and Cas12a nucleases for targeted mutagenesis in maize

Table S4. List of off-targets identified by CIRCLE-seq and Cas-OFFinder.

Off-target	Chr.	OFF-target position	Strand	# mis-matches	Gap	CIRCLE-seq read	Off-target sequence	F primer	R primer	Sequencing primer	Sequencing ID	
9	CIRgRNA2-OFF9	chr8	114426202	+	7	132	AAAGGGGCATAGATCCTGGTGGT	NA	NA	NA		
10	CIRgRNA2-OFF10	chr3	80437794	+	5	122	AAGGGTCGTAGGTCATGAGTGG	NA	NA	NA		
11	CIRgRNA2-OFF11	chr1	71431596	-	4	120	AAGTGGGTACAGTTCATGAGGGG	NA	NA	NA		
12	CIRgRNA2-OFF12	chr9	43647126	-	7	118	GCATGGGCGCAGATCATGGAGGT	NA	NA	NA		
13	CIRgRNA2-OFF13	chr9	29274484	+	6	114	GCGTAGGCCAGATCATGAGGAG	CATCGCGCCTTCTCATCTAT	CTGGTGCCTTTTCCCTCTG	TAATGAGTGGGACCCTCTGG	gRNA2-OFF4	
14	CIRgRNA2-OFF14	chr5	65412390	-	7	96	TCCTGGGTGCAGACCCTGCGGAG	NA	NA	NA		
15	CIRgRNA2-OFF15	chr7	13681611		3	-1	92	TTGTGGGGCAGATC-TGAGCGG	CCTTCCCTCTTCTGGTCTCC	AGGATCCGCATCTCCTTCTT	CGCTCTCAGATCCCTCTCC	gRNA2-OFF5
16	CIRgRNA2-OFF16	chr4	89100310	-	4	90	AAGCGGTGCAGAACATGAGAGG	TTTATCAGTTCGGGTCGAT	GAAAGCTCCCTCGGAACTC	GCTCAAGCCACGATGTATCA	gRNA2-OFF6	
17	Target	chr2	8112430	-	0	86	AAGTGGGCGCAGATCCTGAGCGG	CACAGCCTTGCAATCAATTC	GCTGACGTGGAAGGAGTAGC	ACACCGTGTCTTCGTCAAAA		
18	CIRgRNA2-OFF17	chr9	53910322	-	4	80	AGGGGGTGCAGATCGTGAAGG	GCCTGCTTAGCTCGCATG	TCAAGTCTCCCGTGGAGTT	CATACTTGCCCGCATCTAT	gRNA2-OFF7	
19	CIRgRNA2-OFF18	chr4	131654621	-	4	74	TAGTGGGCACGGATCCCGAGTGG	NA	NA	NA		
20	CIRgRNA2-OFF19	chr8	124038726	-	7	72	TTGTAGGGACAGATCCTGACGGC	CCAAAAGGGGTGGAGTTT	CCGTTTACAGTAGGAGCATT	TGGCCAGGCCTTATTTGTTA	gRNA2-OFF8	
21	CIRgRNA2-OFF20	chr10	10741673	-	4	-1	68	ATGTGGGCACAGATCCAT-GGGG	NA	NA	NA	
22	CIRgRNA2-OFF21	chr5	99843540	+	4	-1	64	AGGTAGGCTCAGATCCT-ATTGG	TCTGATGATGGCATTCTCTGA	GCTTTAGCACCTGGAAAGCA	GAGAAGCATTCGTTTATTGTC	gRNA2-OFF9
23	CIRgRNA2-OFF22	chr2	143394415	+	4	-1	60	GTGGAGGCGCAGATCCTG-GAGG	NA	NA	NA	
24	CIRgRNA2-OFF23	chr8	3233767	-	5	58	GTGTGGGTGCATATCCTGACGGG	NA	NA	NA		
25	CIRgRNA2-OFF24	chr4	102327148	+	6	52	AGTTGGACGCGGAGCCTGAGGAG	CAGGGTTATTGGTGGGAGTG	ACTACTCGCGGATGGAGTTG	AAGAGGGACCATCTGCTGTG	gRNA2-OFF10	
26	CIRgRNA2-OFF25	chr4	120978466	+	3	-1	48	AA-TGGGTGCAATCCTGGGAGG	GGGAGTGTACGCAACTCTCTA	TGGCTGCTTACTACTGTAC	TGGGGTATTGGTTTATCC	gRNA2-OFF11
27	CIRgRNA2-OFF26	chr8	35653730	-	7	48	CTGTGGGCACAGATCCATGGGGA	NA	NA	NA		
28	CIRgRNA2-OFF27	chr5	71309072	-	6	46	TTGTGGACACAGATCCCTAGGGG	NA	NA	NA		
29	CIRgRNA2-OFF28	chr10	2615234	-	5	44	AGCGGGCACAGTCTGAGGGG	GGTTTGCAGAGTGGTACT	CCAGGATGGCTATGAAGCTC	GTGAAGTCCCTGGACGAATG	gRNA2-OFF12	
30	CIRgRNA2-OFF29	chr3	37735259		4	-1	44	CAGTGGGTGCCAATCCT-AGTGG	TTTGCAGCCTCGTGTGCTA	TCCAAGTCCACACTCTOCT	CAACCATGCCACATGAGTA	gRNA2-OFF13
31	CIRgRNA2-OFF30	chr8	45766735		3	-1	40	GAGAGGGGCAGATCC-GAGCGG	NA	NA	NA	
32	CIRgRNA2-OFF31	chr3	22392038	+	4	38	AAGTGGGCAGATTCATGAGGGG	NA	NA	NA		
33	CIRgRNA2-OFF32	chr3	77520472	-	7	34	TCGTGAGTCCAGATCCTGACGGC	AATTTTGGTTTCTCGGATCG	ATACCCCTCCGTTTGTCT	TCGGATCGCGTACTACTACA	gRNA2-OFF14	
34	CIRgRNA2-OFF33	chr5	104806341	-	3	34	TAGTGGGCGCGAGCCTGAGTGG	NA	NA	NA		
35	CIRgRNA2-OFF34	chr5	34959898	-	4	34	AAGTAGGCGGAGTTCATGAGGGG	NA	NA	NA		
36	CIRgRNA2-OFF35	chr8	28495318	-	5	34	AGGTGGGCATAGATGCTGAGGCG	NA	NA	NA		
37	CIRgRNA2-OFF36	chr10	31243099	-	3	32	AAGTGGGCGCAGTCTCGGCGG	NA	NA	NA		
38	CIRgRNA2-OFF37	chr4	146758923	-	3	-1	32	AGGAGGGCGCAGATC-TGATGGG	NA	NA	NA	
39	CIRgRNA2-OFF38	chr5	122305522	+	4	-1	32	ATGTGAGGGCAGATCCCG-GCGG	GCTAATAAATTTCCCGCAACG	TGGATAGACTGGACTGGATGG	GAGACCGACGGACCTGTAAA	gRNA2-OFF15
40	CIRgRNA2-OFF39	chr9	44365953	+	4	32	TAGCGGGCGGAGATCCTGCGGGG	NA	NA	NA		
41	CIRgRNA2-OFF40	chr3	33755704	+	3	30	TAGTGGGCGCGAGCCTGAGTGG	NA	NA	NA		
42	CIRgRNA2-OFF41	chr4	31939351		4	-1	30	CAGTGGGCAAGATC-TGAGCGG	NA	NA	NA	
43	CIRgRNA2-OFF42	chr1	72856206	-	3	28	TAGTGGGCGCGGAGCCTGAGTGG	NA	NA	NA		
44	CIRgRNA2-OFF43	chr5	126727666	-	2	28	TAGTGGGCGCAGACCTGAGTGG	NA	NA	NA		
45	CIRgRNA2-OFF44	chr7	46673844	+	4	-1	28	TA-TGGGCACGGATCCTGGGTGG	NA	NA	NA	

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Table S4. List of off-targets identified by CIRCLE-seq and Cas-OFFinder.

Off-target	Chr.	OFF-target position	Strand	# mis-matches	Gap	CIRCLE-seq read	Off-target sequence	F primer	R primer	Sequencing primer	Sequencing ID	
46	CIRgRNA2-OFF45	chr1	42186271	+	3	26	TAGTGGGCGCGGATCCCGAGTGG	NA	NA	NA		
47	CIRgRNA2-OFF46	chr6	3885198	-	5	24	CGGTCGGCGCAGATCCGAAGGG	AGCTCAGGTACACGCTGCTC	CGGGTCTTTCTGGTCTTCAA	GAGCTCAAACGCTTCGCTAT	gRNA2-OFF16	
48	CIRgRNA2-OFF47	chr6	19911665	-	4	22	AAGTGGGCGAGATTCATGAGGG	NA	NA	NA		
49	CIRgRNA2-OFF48	chr8	78599198	+	3	22	TAGTGGGCGCGGAGCCTGAGTGG	NA	NA	NA		
50	CIRgRNA2-OFF49	scaffold5.1481	2130	+	5	290	ATATGGGAGAAGATCCTGAGGGT	TCACGAAAGGAACGAAAGGT	TTTTCCATGGAGCCAGATTC	CGATGAGCACCAGTCATTGT	gRNA2-OFF19	
51	CIRgRNA2-OFF50	scaffold7.1524	51025	+	6	168	TGGTGGGCCAGATCATGATGGA	NA	NA	NA		
52	CIRgRNA2-OFF51	scaffold5.1556	182655	+	7	134	CCGAGGGCGCAAATCCTGGTGGT	NA	NA	NA		
53	CIRgRNA2-OFF52	scaffold5.1846	47657	+	6	100	GAGGGGGCGCAGCTCCTAGGGGC	NA	NA	NA		
54	CIRgRNA2-OFF53	scaffold1.2368	3376	+	7	58	CTCAAGGCGCAGATCGTGAGCG	NA	NA	NA		
55	CIRgRNA2-OFF54	scaffold7.2180	31663	-	4	-1	58	TTGAGGGCGCAGATC-TGTGAGG	GTGTCCCTGTTTCCATGACC	ACATAGCCCTCCCTAGCAT	GGGTGTTAGGGAGGAGAAG	gRNA2-OFF20
56	CIRgRNA2-OFF55	scaffold7.857	39835	-	7	56	CTGTGGACACAGATCCTTGGGGA	NA	NA	NA		
57	CIRgRNA2-OFF56	scaffold7.361	17864	+	4	54	TAGTGGGCGCGGATCCCAAGTGG	NA	NA	NA		
58	CIRgRNA2-OFF57	scaffold5.414	18095	+	6	52	GCGGAGGCGCAGATCCCGAGCAG	NA	NA	NA		
59	CIRgRNA2-OFF58	scaffold7.575	108704	+	4	42	AAGTGGACGGAGCTCATGAGGGG	NA	NA	NA		
60	CIRgRNA2-OFF59	scaffold1.3684	8306	+	3	36	AAGTGGGCGGAGTTCATGAGGGG	NA	NA	NA		
61	CIRgRNA2-OFF60	scaffold9.2352	20721	-	7	36	TTGCAGGCGAAGACCCTGAGCAG	NA	NA	NA		
62	CIRgRNA2-OFF61	scaffold10.306	139567	-	6	34	GAATGGACACAGATCCTGAGGAG	NA	NA	NA		
63	CIRgRNA2-OFF62	scaffold4.1476	188749	+	7	32	ATGTGGACACAGATCCATGGGGA	NA	NA	NA		
64	CIRgRNA2-OFF63	scaffold3.2145	3787	+	6	30	GAGGGAGCGCAGATCACGGGGG	NA	NA	NA		
65	CIRgRNA2-OFF64	scaffold3.903	101030	+	3	30	TAGTGGGCGCAGAGCCCGAGTGG	NA	NA	NA		
66	CIRgRNA2-OFF65	scaffold4.843	7341	+	4	28	AAGTGGGCGAGTTCATGAGGGG	NA	NA	NA		
67	CIRgRNA2-OFF66	scaffold5.1936	55512	+	4	28	AAGTGGGCGAGTTCATGAGGGG	NA	NA	NA		
68	CIRgRNA2-OFF67	scaffold2.1507	228125	+	4	-1	26	GTGAGGAGCAGATC-TGAGCGG	NA	NA	NA	
Cas9-gRNA2: Cas-OFFinder												
1	CASgRNA2-OFF1	chr1	35799146	-	2	-1	ACGTGGGCGCAATCCTGCGCGG	TAGGCAGACAATGAGCCAAA	TAGATCGAGAAGGCGTCTG	CCAAACTAACCTGAGCGATCT	gRNA2-OFF17	
2	CASgRNA2-OFF2	chr1	73355715	+	2	-1	ATGTGGGCGCAATCCTGCGTGG	NA	NA	NA		
3	CASgRNA2-OFF3	chr1	93320177	-	2	-1	ACGTGGGCGCAATCCTGCGCGG	NA	NA	NA		
4	CASgRNA2-OFF4	chr10	31243108	-	3		AAGTGGGCGCAGTCTCTGGCGG	CCGCAGATAAGACACTGACG	GTGACGTGGAAGGAGAAGGA	CAAGACGAGTGTCTGCTAA	gRNA2-OFF18	
5	CASgRNA2-OFF5	chr1	73355694	-	2	-1	ATGTGGGCGCAATCCTGCGTGG	NA	NA	NA		